

ARTIFICIAL LONG TERMINAL REPEAT VECTORS

ABSTRACT OF THE DISCLOSURE

Disclosed are compositions and an in vitro method for cloning and/or amplification of nucleic acid sequences of interest. The method is based on strand displacement replication of the nucleic acid sequences by multiple priming on artificial long terminal repeat (ALTR) sequences appended to the ends of the nucleic acid molecule of interest. The nucleic acid molecules for cloning and amplification can be very long, up to 40 to 80 Kb or longer. In a preferred form of the method, a single primer is used to prime strand displacement replication at multiple sites in artificial long terminal repeat sequences, flanking a target nucleic acid, containing multiple tandem repeats of a primer complement sequence. Amplification proceeds by replication initiated at each primer and continuing through the target nucleic acid sequence. This nested replication of multiple copies significantly increases the amplification yield for extremely long nucleic acid molecules. By using a sufficient number of repeat units in the ALTRs, only a few rounds of replication are required to produce hundreds of thousands of copies of the nucleic acid sequence of interest. A preferred form of the disclosed method makes use of indexed artificial long terminal repeats that allow amplification and identification of specific nucleic acid fragments present in a mixture of nucleic acid fragments without requiring any knowledge of the sequence of the nucleic acid fragment.